Title:

Aposematism: balancing salience and camouflage

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Abstract

Aposematic signals are often characterised by high conspicuousness. Larger and brighter signals reinforce avoidance learning, distinguish defended from palatable prey, and are more easily memorised by predators. Conspicuous signalling, however, has costs: encounter rates with naïve, specialised, or nutritionally stressed predators are likely to increase. It has been suggested that intermediate levels of aposematic conspicuousness can evolve to balance deterrence and detectability, especially for moderately defended species. The effectiveness of such signals, however, has not yet been experimentally tested under field conditions. We used dough caterpillar-like baits to test whether reduced levels of aposematic conspicuousness can have survival benefits when predated by wild birds in natural conditions. Our results suggest that, when controlling for the number and intensity of internal contrast boundaries (stripes), a reduced-conspicuousness aposematic pattern can have a survival advantage over more conspicuous signals, as well as cryptic colours. Furthermore, we find a survival benefit from the addition of internal contrast for both high and low levels of conspicuousness. This adds ecological validity to evolutionary models of aposematic saliency and the evolution of honest signalling.

Key words

aposematism; camouflage; defensive colouration; honest signalling; visual signalling; warning signals.

1. Background

In order to escape predation, chemically defended species often signal their unpalatability with conspicuous colour patterns [1-3]. Predators learn to associate colouration and unprofitability, with increasing conspicuousness often increasing the speed and longevity of avoidance learning in avian predators [2-4]. By raising contrast against the background, aposematic patterns increase distinctiveness from palatable prey, which are often camouflaged, and become more easily recognised when subsequently encountered [5-7]. In many aposematic patterns high contrast boundaries also extend across the body, with bright colours frequently combined with patches of black. These internal contrast boundaries have received comparatively little attention but may act to increase the saliency of signals and/or promote signal constancy across heterogeneous backgrounds and light conditions [3; 4].

Conversely, although greater detectability can improve the efficacy of aversive signalling, high levels of conspicuousness can lead to more encounters with naïve or specialised predators which may ignore the warning [8; 9]. Variation in predator reactions to defended prey also occurs intra-specifically and temporally as individual predators manage their own toxin burden and nutritional requirements [10; 11]. For intermediately defended species maximising conspicuousness may not maximise survival and, instead, animals should balance signal efficacy with predator encounter rates [10-12]. It has been suggested that pattern elements, in addition to promoting recognition and memorability, can interact with the background and one another to reduce detectability [13-17]. However, the role of pattern, rather than colour saturation, in reducing detectability and signalling defence strength has not been investigated in much detail [3].

Previous theoretical and laboratory work has shown that maximising detectability may not maximise survival [8; 11; 12; 14-17]. We used artificial caterpillars and free-living wild passerine birds to investigate whether intermediate levels of conspicuousness are effective in the field.

2. Methods

(a) Stimuli

The experiment followed a well-established paradigm with wild avian predators selectively predating dough, caterpillar-like, baits [18]. Stimuli were ~16mm long (~3mm wide) cylinders of dough, coloured to produce notionally camouflaged and aversive patterns. Seven treatments were used, designed to vary in conspicuousness while controlling for internal contrast boundaries. Treatments were either predominantly yellow (a common component of aposematic colouration) and highly conspicuous, mostly black (an inconspicuous colour for the backgrounds used, and often associated with aposematic patterns) or various mixtures of the component colours, appearing olive-green to the human eye (figure 1). High conspicuousness treatments were Y_P (plain yellow) and Y_S (yellow with thin black stripes (3:1)). Low conspicuousness treatments were B_P (plain black) and B_S (black with thin yellow stripes (3:1)). The average mixtures were Y_A (3:1 mix of yellow and black), B_A (3:1 mix of black and yellow), and A_V (1:1 yellow-black mix). The difference in colour contrast between the treatments and the background was verified by avian colour space modelling (Supplementary Material).

Dough was made from a 3:1 mix of flour (British Plain Flour by Sainsbury's, J Sainsbury plc.) and lard (Sainsbury's Basics Lard, J Sainsbury plc.), which was then coloured yellow (25ml per 500g dough; Yellow Food Colouring by Sainsbury's, J Sainsbury plc.), or black (25ml per 500g dough; Black Food Colouring by Sainsbury's, J Sainsbury plc.). Different ratios (see above) of coloured dough were then thoroughly mixed to create average colour treatments (Y_A, B_A, and A_V). All 'caterpillars' were then built from 16 disks of dough, 3mm in diameter and 1mm thick.

(b) Survival protocol

Between October and March, 15 blocks of 70 dough caterpillars (10 x seven treatments per block; n = 1050) were run in areas of suburban green space in and around the city of Bristol, UK. 'Caterpillars' were pinned along non-linear transects to the horizontal stems of bramble plants (*Rubus fruticosus* agg. Rosaceae), at a height of ~1.5m, and were unobscured by surrounding vegetation. The survival of ‘caterpillars’ was recorded at 24, 48, 72, and 96 h, with the mortality rate analysed with a mixed effects Cox model (package *coxme* [19] in R 3.1.3 with *block* as a random factor). Contrasts of *a priori* interest (striped vs plain and average equivalents) were tested without controlling for multiple testing as the number (6) is less than the degrees of freedom for treatment [20]; all other comparisons used the False Discovery Rate to control Type I error using R package *multcomp* [21]. Evidence of avian predation (beak marks or complete removal) was recorded as a terminal event, whereas all other forms of ‘mortality’, including predation by other species (gastropods and Hymenoptera, identified by slime trails and small pit marks respectively), missing pins, broken baits, and survival to 96 h, were included as censored values.

3. Results

In total 569 of the 1050 ‘caterpillars’ (54%) were predated by birds. Treatment affected survival (χ^2 = 27.53, d.f. = 6, $p < 0.001$) and so pairwise comparisons were performed (figure 2). There was a significant survival increase resulting from stripe addition for both low conspicuousness (B_S-B_P: z = 3.72, $p < 0.001$) and high conspicuousness (Y_S-Y_P: z = 2.52, p = 0.012) patterns. When compared to their average colours, low conspicuousness stripes (B_S) had significantly greater survival (B_S-B_A: z = 3.74, $p < 0.001$), whereas high conspicuousness stripes (Y_S) survived similarly to their corresponding average colour (Y_S-Y_A: z = 0.53, p = 0.600). The low conspicuousness (B_S) striped pattern had higher survival than the high conspicuous (Y_S) striped treatment (B_S-Y_S: z = 2.22, p = 0.026) and the 1:1 average (B_S-A_V: z = 2.78, p = 0.006). We found no significant difference between any other post hoc comparisons (all $p > 0.284$).

4. Discussion

The observed survival patterns lead to two conclusions: i) the addition of internal contrast boundaries (stripes) can increase survival regardless of the base colour’s conspicuousness ($Y_S > Y_P$ and $B_S > B_P$); and ii) intermediate levels of conspicuousness (B_S) can survive better than both high conspicuousness (Y_S) and camouflage (A_V, B_A, and B_P). This has implications for the evolution of aposematic conspicuousness under ecologically relevant multi-species predation risk.

Patterns which were predominantly black but contained thin yellow stripes (B_S) had a survival advantage over yellow patterns with thin black stripes (Y_S), even though the number and intensity of internal pattern boundaries was equal. Prior experience with a natural, aversive, B_S-like prey cannot explain this result: there are no common caterpillars in the study area with patterns like those used in this study. This suggests that a lower level of detectability can increase survival despite potentially compromising the degree of aversion. This low conspicuousness striped pattern (B_S) also had a survival advantage over plain patterns B_P and B_A, demonstrating that a failure to detect the stripes is not the sole driver of this effect. Instead, we propose that this pattern occupies a fitness peak corresponding to a low level of detectability at a distance, backed up by an effective aposematic signal close-up [14-17].

Furthermore, we find that adding highly contrasting stripes (either yellow or black) to otherwise homogenously coloured stimuli can increase survival regardless of the initial detectability of the pattern. The effect of pattern appears to be separate from the effect of conspicuousness, and plausibly lies in aversion. The role of pattern is contentious, with some authors reporting that its presence acts as an aversive signal when combined with [22-23] or in the absence of conspicuous colouration [24-26], whereas others have found conflicting results [4; 27].

Under natural levels of heterogeneity camouflage and aposematism are both likely to be undermined by diversity in predator reactions and the visual environment. Previous studies have suggested that intermediate levels of detectability may act to combine camouflage and aposematism as a function of observer distance [14-17]. Our results corroborate these findings under field conditions and suggest that these patterns can indeed provide increased survival compared to full investment in conspicuousness or camouflage. Manipulating pattern can be an effective mechanism of reducing the detectability of aposematic signals, adding ecological validity to suggestions that intermediate levels of conspicuousness can be evolutionarily stable.

Ethics. Experiments were approved by the University of Bristol Animal Welfare and Ethical Review Body.

Data accessibility. Raw data can be accessed from the Dryad data repository at [doi to be added].

Authors' contributions. J.B.B. collected the data, and all authors participated in experimental design, analysis, and writing of the manuscript. All authors gave final approval for publication and agreed to be accountable for all aspects of the content therein.

Competing interests. We have no competing interests.

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Figure legends

Figure 1. Dough caterpillar treatment designs.

(a) top to bottom, and (b) clockwise from top-left: Y_P – high conspicuousness yellow; B_P – low conspicuousness black; A_V – average colour control and reference treatment (1:1 ratio yellow-black); B_A – average colour of B_S (1:3 yellow-black); B_S – low conspicuousness with stripes (1:3 yellow-black); Y_A – average colour of Y_S (3:1 yellow-black); Y_S – high conspicuousness with stripes (3:1 yellow-black).

Figure 2. Relative survival of defensive patterns (odds ratios compared to treatment A_V, with 95% CI from model). The low conspicuous aposematic pattern (B_S) has a higher survival than the more cryptic patterns (A_V, B_P, and B_A) and the more conspicuous striped pattern (Y_S). The addition of contrasting stripes increases survival for both inconspicuous ($B_S > B_P$) and conspicuous ($Y_S > Y_P$) patterns.

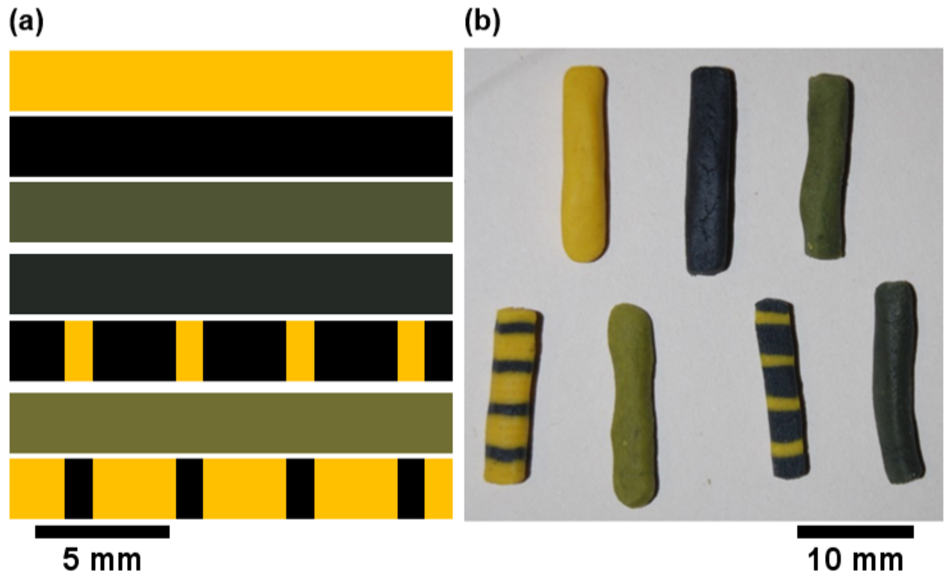

Figure 1

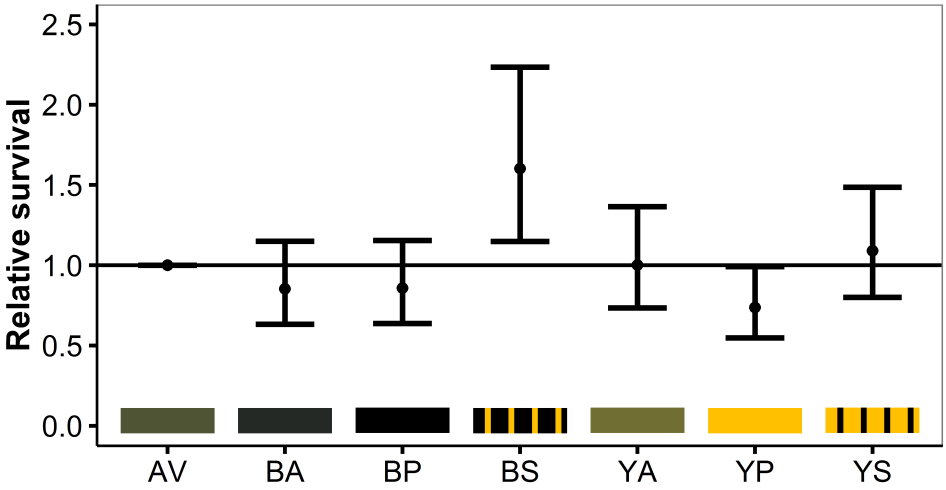

Figure 2

Supplementary material

Aposematism: balancing salience and camouflage.

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S1. Image analysis

As many avian predators can detect ultraviolet (UV) light, dough caterpillars were photographed with a UV sensitive Nikon D70 digital camera (Nikon Corporation, Tokyo, Japan) and UV-VIS 105mm CoastalOpt® SLR lens (Jenoptik AG, Jena, Germany) under natural, clear, daylight conditions. This revealed minimal UV reflectance from the yellow and black dough, as well as their blended colours (figure S1a-b).

The lack of UV reflectance allows avian vision to be modelled from standard, but calibrated, photographs. The use of calibrated photographs rather than spectrometry allows us to categorise the true visual scene which includes areas of shadow and visual texture not picked up in point source reflectance measurements.

The UV sensitive tetrachromatic vision of the European starling (*Sturnus vulgaris*, Sturnidae) has four single cones, with peak sensitivities (λ_{max}) of 563nm (L), 504nm (M), 449nm (S), and 362nm (UV), and luminance measuring double cones (D) with λ_{max} of 563nm [28]. As there was negligible UV reflectance from the dough caterpillars, colour perception was modelled in trichromatic space as a product of luminance (L), and the opponent channels red to green (rg), and yellow to blue (yb). Luminance was measured directly by the response of double cones, the red to green opponent channel was produced from the relative stimulation of the longwave (L) cone and the mediumwave cone (M), and the yellow to blue channel was produced by combining the mean stimulation of the longwave (L) and mediumwave (M) cones to the shortwave cone (S). The rationale for transforming the S, M and L cone inputs to S vs M+L (i.e. yellow-blue) and M vs L (i.e. red-green) outputs was that (i) this creates two contrasts that are orthogonal (in the sense of statistical independence) and (ii) these capture the main variation in the colours involved (which are black, yellow, and green). Modelling the black, green, and yellow colours in a hypothetical colour opponent system containing all possible combinations -- S vs M, S vs L, M vs L, (S+M) vs L, S vs (M+L) and M vs (S+L) contrasts -- would give the same results (because these are all mappings from the same photon catch data), but rather less efficiently because most of these 'dimensions' are redundant. We are not claiming that starlings do have yellow-blue or red-green opponency, just that they have colour opponent channels that achieve the same effect.

Ten photographs of dough colour (Y_P, B_P, A_V, B_A, B_S, Y_A, and Y_S) were taken with a Nikon D3200 digital camera (Nikon Corporation, Tokyo, Japan), from a distance of ~50cm and at a 45° angle (figure S1c-i). Each image contained a ColorChecker Passport (X-Rite Inc., 2009. MI, USA) which allowed colour calibration, linearization, and appropriate scaling. Of these 61 were suitable for analysis (A_V = 10, B_A = 8, B_P = 9, B_S = 9, Y_A = 7, Y_P = 9, Y_S = 9), each photograph was calibrated, and the coordinates corresponding to the 'caterpillar' and the background were specified in MATLAB 2015a (The MathWorks Inc. MA, USA).

Plotting the model's response to the background, for each dough colour used to produce the 'caterpillars', shows that whereas the yellow dough (Y_P) is an obvious outlier from the background, all other colours (A_V, Y_A, B_P, and B_A) are well represented in the background (figure S2). This adds weight to the assertion that yellow was a conspicuous colour in this environment, and that the other colours can produce effective camouflage to an ecologically relevant avian predator.

S2. Supplementary references

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Figure S1. Dough caterpillar designs and stimuli photographed *in situ* on bramble stems, as used for image analysis.

Examples of treatment designs. (a) top to bottom, and (b) left to right: Y_P – plain yellow; B_P – plain black; A_V – 1:1 average of yellow and black; Y_S – yellow with black stripes (3:1 yellow-black); Y_A – 3:1 average of yellow and black; B_S – black with yellow stripes (1:3 yellow-black); B_A – 1:3 average of yellow and black.

Examples of stimuli *in situ*. (c) A_V; (d) Y_P; (e) Y_S; (f) Y_A; (g) B_P; (h) B_S; (i) B_A.

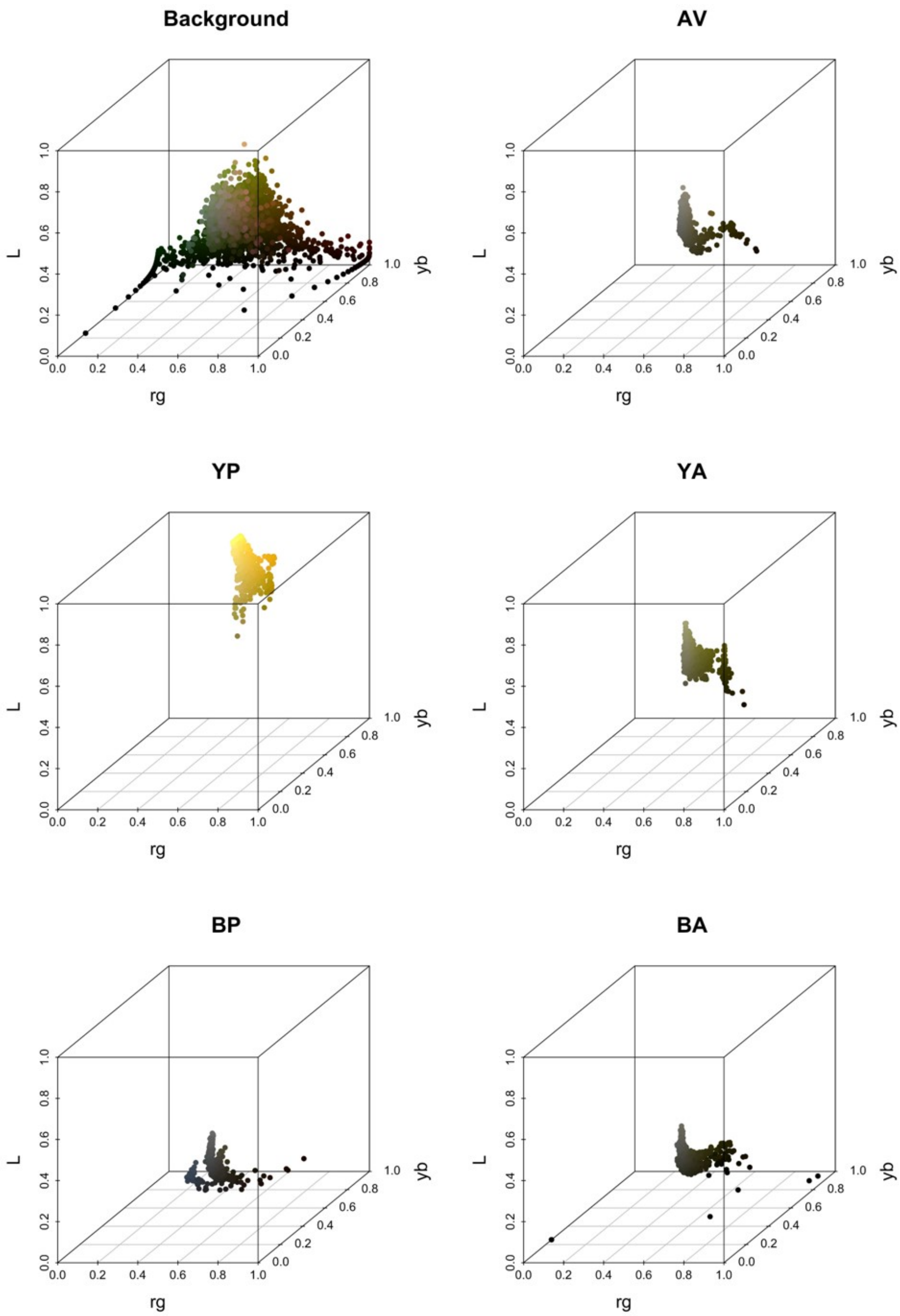

Figure S2. Dough caterpillar colours as seen by a model of avian vision. All 'caterpillar' colours are well represented as a subset of the background colours, except for yellow (Y_P) which differs in both colour and luminance.